MOLECULAR DOCKING ANALYSIS OF *Aerva lanata* PHYTO CONSTITUENTS AS LEAD FOR MICROBIAL INHIBITORS

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ABSTRACT

Molecular docking study was performed using Maestro Schrödinger suite 8.5 mainly on twenty nine phytoconstituents reported from the plant Aerva lanta for their antimicrobial potential. The crystal structure of protein data bank (PDB-ID: 3SRW) was obtained from RCSB (Research Collaboratory for Structural Bioinformatics) website. The ligands were obtained from the reported literature search of Aerva lanta plant. The top hits were analyzed for their binding affinity with the dihydrofolate reductase enzyme. The docking results revealed that rutin (Glide score: -11.75) exhibited better binding interaction to dihydrofolate reductase receptor.

Keywords: *Aerva lanata*, Molecular docking, antimicrobial, dihyrofolate reductase

INTRODUCTION

Natural goods have gained a lot of significance since ancient times due to their lesser side effects, higher safety and efficacy against health illness. In current era, plants have screened for their potential uses as an alternative medicines to allopathic medicines for the treatment of many diseases. The lesser side effect of natural products is due to their natural antioxidant properties.[1] As a result, it is essential to unlock their potential for the development of newer drugs against health risks.

Over the past several years, the increase of bacteria drug-resistance and the rapid emergence of new infections have intensely decreased the efficiency of the drugs against pathologies caused by certain microorganisms. This situation rises up the urgent need for

the development of new antibacterial agents, preferentially, from natural sources.[2] The active principle of bioactive compounds has shown tremendous therapeutic applications either singly or in combination to inhibit the life processes of microbes.

Aerva lanata (Linn.) Juss. Ex Schult. belongs to the family *Amaranthaceae*, is one of the important plant grow in the warmer parts of India ascending to 1,000 m. In Sanskrit *A. lanata* is known as paashaanabheda, gorakshaganjaa, satkabhedi, aadaanpaak. It is commonly known as sirupeelar in Tamil or Siddha.[3] The plant is extensively used in urinary disorders like *Ashmari* (Urinary calculi), *Mootrakrichra* (Dysuria), *Mootravikara* etc by most of the Ayurveda and Siddha practitioners in southern India, in the name of *Pashanabheda*. As the plant bears almost all the properties similar to that of the original source of *Pashanabheda*.[4] Herbs are perennial, 5–50 cm tall. Stem branched from base; branches ascending or stoloniferous, white lanose. Leaves opposite or nearly whorled, sessile, grayish green, subulate, linear, 1–2.5 cm, abaxially white lanose, adaxially glabrous, base attenuate, sometimes vaginate.[5,6] Flowers are small in size, sessile, greenish or dull white in colour, clustered with spikes. The phytoconstituents reported from *Aerva lanata* plant are flavanoids, tannins, anthra-quinons, alkaloid, phenol, proteins, amino acids and carbohydrates.[7]

However, from literature reviewed till date, it is obvious that there is no information available about the *in silico* antimicrobial activity of phytoconstituents from *Aerva lanata*. The present docking studies was done to explore the lead molecules from *Aerva lanata* for antimicrobial activity.

MATERIALS AND METHOD

Molecular docking simulation was performed using Glide module in Maestro 8.5 version software (Schrodinger LLC suite). Schrodinger suite was installed in a system having configurations core^{TM} processor with 2 GB RAM and 320 GB ROM with CentOS Linux as the operating system.

Protein Preparation

The X-ray crystal structure of antimicrobial target protein (PDB-ID: 3SRW) was accessed from RCSB protein data bank.[8] The crystal structure of dihydrofolate reductase enzyme receptor was

reported to complex with 7-(2-ethoxynaphthalen-1-yl)-6-methylquinazoline-2,4-diamine. Protein preparation was performed using Protein Preparation wizard in Maestro. In this step, water molecules were removed, bond orders were assigned, all hydrogen's in the structure were added, and bonds to metals were deleted and the formal charge in the metal & neighboring atoms were adjusted that more than the 5Å specified distance. The next stage is to inspect and change the protonation state for the residue in the workspace for minimal structural errors. The final step in the protein preparation process was to refine the structure, with a restrained minimization. In order to determine the potential binding sites, a grid based cavity prediction algorithm has been used. Protein preparation method follows OPLS-2005 force field for energy minimization.

Ligand Preparation

The ligands were built using Chem Draw Ultra 10.0 converted to 3D structure from the 2D structure using the same software. Chem Draw Ultra 8.0 is a robust collection of tools designed to prepare high quality, all atom 3D structure for large numbers of drugs-like molecules, starting with the 2D or 3D structure in mol format.[9] The resulting structures are saved in Mol format and imported to Maestro project file. The simplest use of Chem Draw Ultra 10.0 is to produce a single, low energy, 3D structure with correct chiralities for each successful proposed input structure. Further steps were performed using LigPrep module in Maestro. While performing ligand preparation step, chiralities were determined from 3D structure and original states of ionization were retained. Tautomers were generated discarding current conformers. The conformational space was searched using the Monte Carlo method. All rotable single bonds were included in the conformational search. Each search was continued until the global energy minima were found at least 10 times. The energy minimizations were carried out using the least square OPLS_2005 force field. The conformational searches were done for aqueous solution using the generalized Born/solvent accessible surface (GB/SA) continuum salvation model.[10]

Grid generation & docking calculation

Glide searches for favorable interactions between ligand molecules and the receptor. Grids were generated using Receptor Grid Generation module in Glide following the standard procedure recommended. Grid generation defined the active sites of the protein and generated the electrostatics grid. Constraints were included in the grid files. The shape and the properties of the

receptor were represented on a grid by several different sets of field that provide progressive more accurate scoring of the ligand poses. Ligand molecule was picked so that it can be excluded from the grid generation with the van der Waals radius scaling 1.00.[10] The ligands were docked using docking functionality with extra precision (XP) mode. The most feasible orientation of the ligands in the binding pocket is predicted, and the strength of the interaction in the particular orientation was quantified from a scoring function.

RESULT & DISCUSSION

The hydrogen and nitrogen atoms of internal ligand were bound with the hydrophobic pocket of the receptor with residue MET 528 and CYS 530 by forming two H-bonds of length 3.091 Å and 3.091 Å having the docking score of **-3.81**. Ligands Rutin, Quercetin, Myrecetin exhibited better binding affinity towards the receptor as compared to other ligands. Rutin showed very good affinity towards the receptor with highest dock score of **-11.75**. It formed two hydrogen bonds of length 2.186 Å and 2.146 Å and oxygen and hydrogen atoms of ligands with ASP 351 residue of receptor.

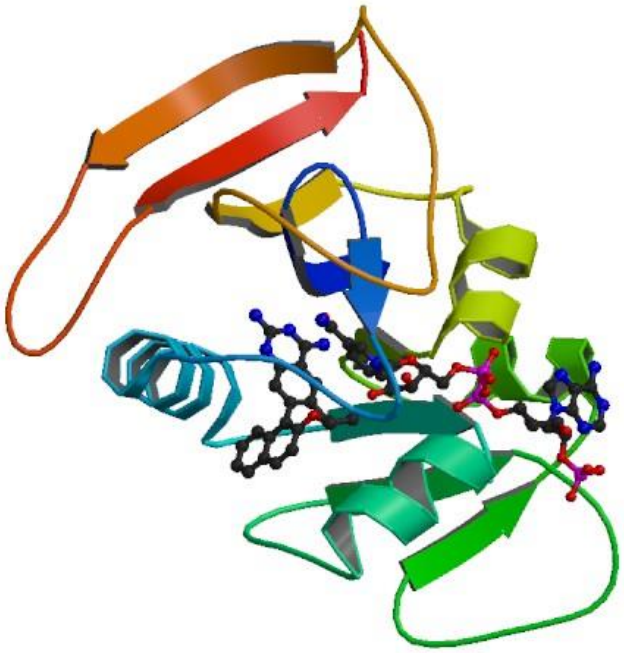
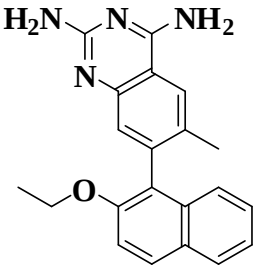
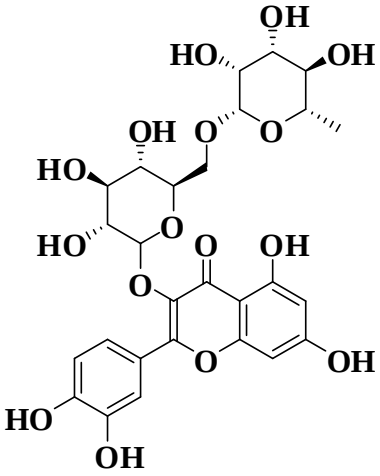
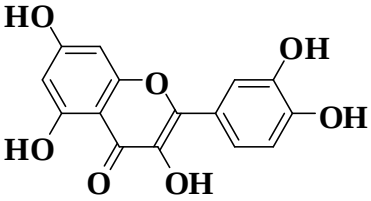
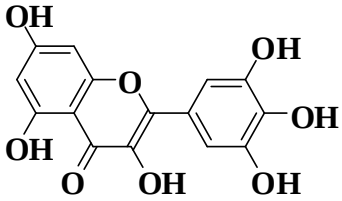
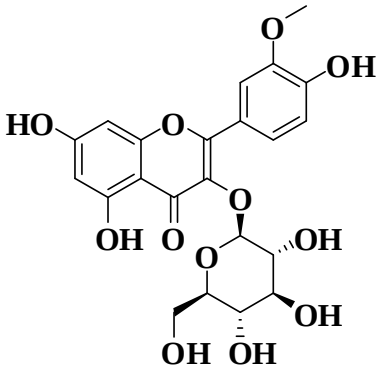
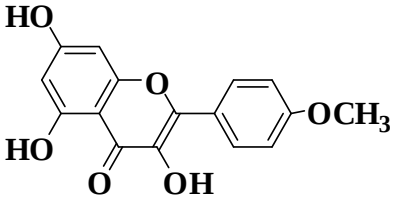
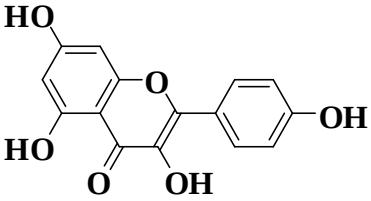
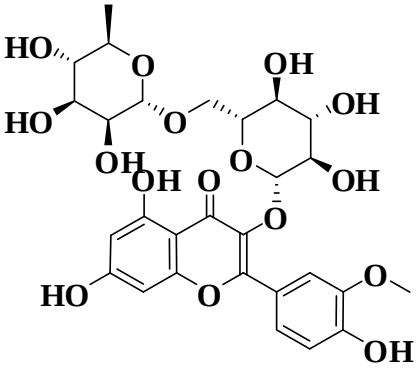
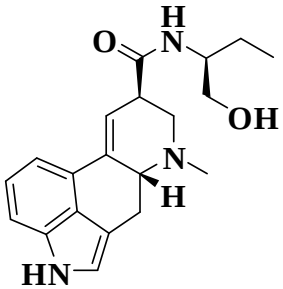
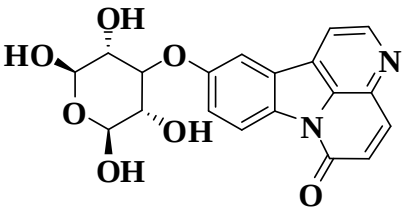
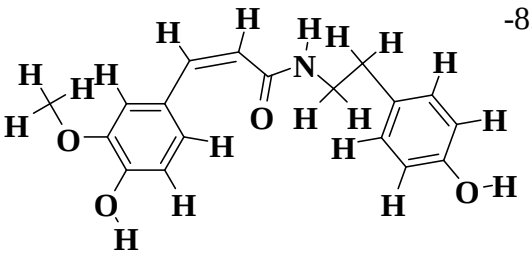
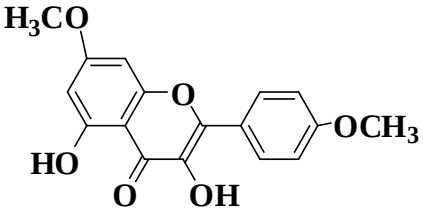
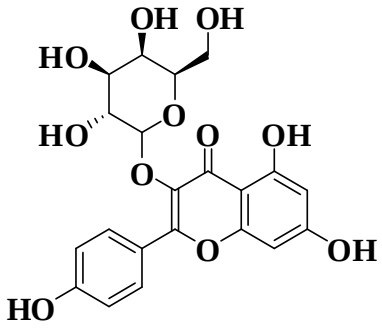
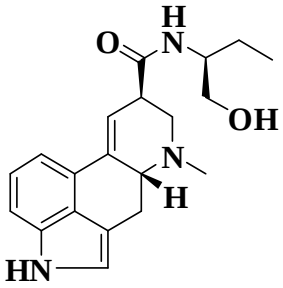
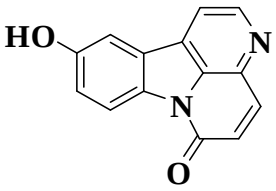
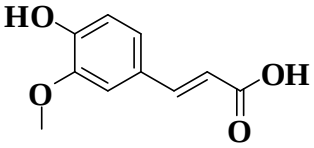
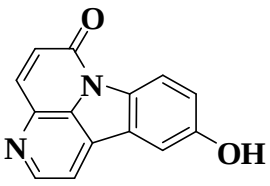


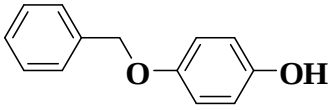
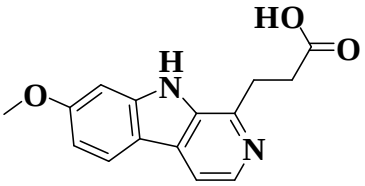
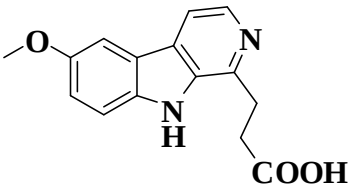
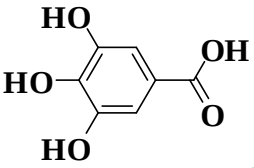
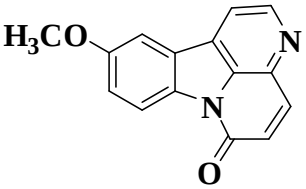
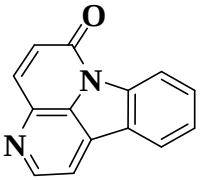
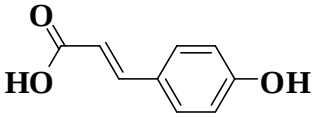
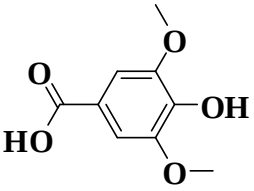
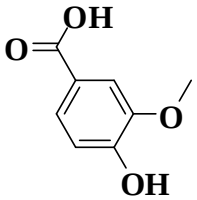
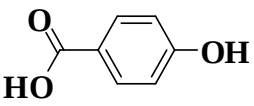
Figure 1: Secondary structure of antimicrobial enzyme receptor (PDB-ID: 3SRW)

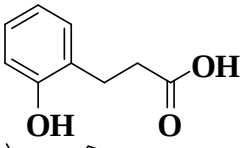
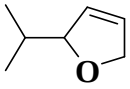
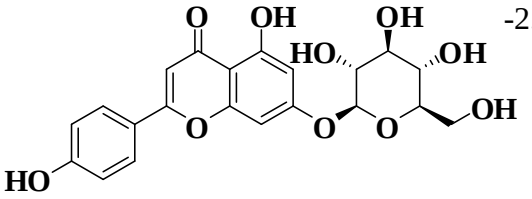
Table1: Docking Score of *Aerva lanata* phytoconstituents with antimicrobial receptor (PDB-ID: 3SRW)

Sr. No.	Ligand Name	Ligand Structure	Docking Score
1.	**Internal Ligand [7-(2-ethoxynaphthalen-1-yl)-6-methylquinazoline- 2,4-diamine]**		**-3.81**
2.	**Rutin**		**-11.75**
3.	Quercetin		-9.60
4.	Myricetin		-9.34

5.	Isorhamnetin		-8.85
6.	4’Mrthoxykaempferol		-8.75
7.	Kaempferol		-8.74
8.	Narcissin		-8.68
9.	Methergine(Methylergometrin)		-8.35
10.	Ervoside		-8.25

11.	Feruloyltyramine		-8.18
12.	4’,7-Dimethoxykaempferol		-8.02
13.	Kaempferol-3-rhamnogalactoside		7.87
14.	Methergine		-7.71
15.	Ervine		-7.60
16.	Ferulic acid		-7.50
17.	Aervine		-7.42

18.	Hydroxyquinone monobenzyl ether		-6.90
19.	3-β-carboline-1-yl-propionic acid		-6.75
20.	Ervolanine		-6.60
21.	Gallic acid		-6.45
22.	Methylervine		-6.20
23.	Canthin-6-one		-5.95
24.	p-Coumeric acid		-5.52
25.	Syringic acid		-5.05
26.	Vanillic acid		-4.84
27.	PHBA (p-Hydroxybenzoic acid)		-4.69

28.	Melilotic acid		-4.69
29.	2-isopropyl-2,5-dihydrofuran		-3.81
30.	Apigetrin		-2.38

Docking analysis is a complex process which involves analysis of various ligand-receptor interactions like hydrogen bonding interactions, hydrophobic interactions, π-π stacking, metal co-ordination etc. Here, in the present work top three hits were analyzed for their receptor interactions as:

1. Internal Ligand [7-(2-ethoxynaphthalen-1-yl)-6-methylquinazoline- 2,4-diamine]

The internal ligand was used as standard ligand to compare docking and scoring function of other ligands. The hydrogen bond interactions of hydrogen and nitrogen atoms of ligand with MET 528 and CYS 530 residues of receptor were seen respectively. The other hydrophobic interactions observed were CYS 530, VAL 533, PRO 535, LEU 536, VAL 534, TRP 383, MET 522, and LEU 539 (Figure 2). The docking score of -3.816025 was calculated by the docking software.

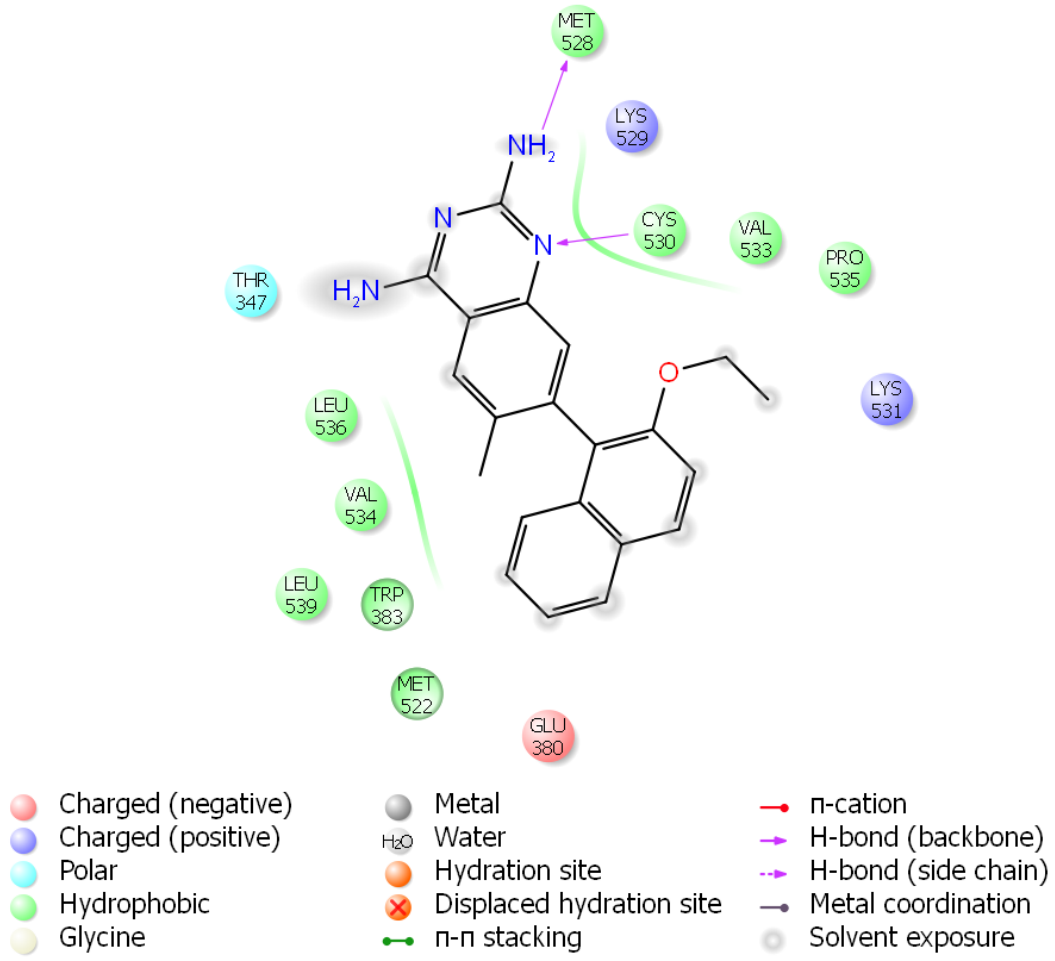


Figure 2: Ligand-recptor 2D interaction diagram of Internal Ligand

[7-(2-ethoxynaphthalen-1-yl)-6-methylquinazoline- 2,4-diamine]

2. Rutin

Overall, rutin showed the highest docking score of -11.75 with notable two hydrogen bonding interactions of hydrogen and oxygen atoms of ligands with ASP 351. Additionally, hydrophobic interactions (ALA 350, LEU 525, TYR 526, MET 522, TRP 383, LEU 536, MET 528, CYS 530, VAL 533, LEU 539, VAL 534, MET 388, LEU 384, LEU 391, LEU 387, ILE 424, MET 421, LEU 346 and MET 343) were too observed (Figure 3).

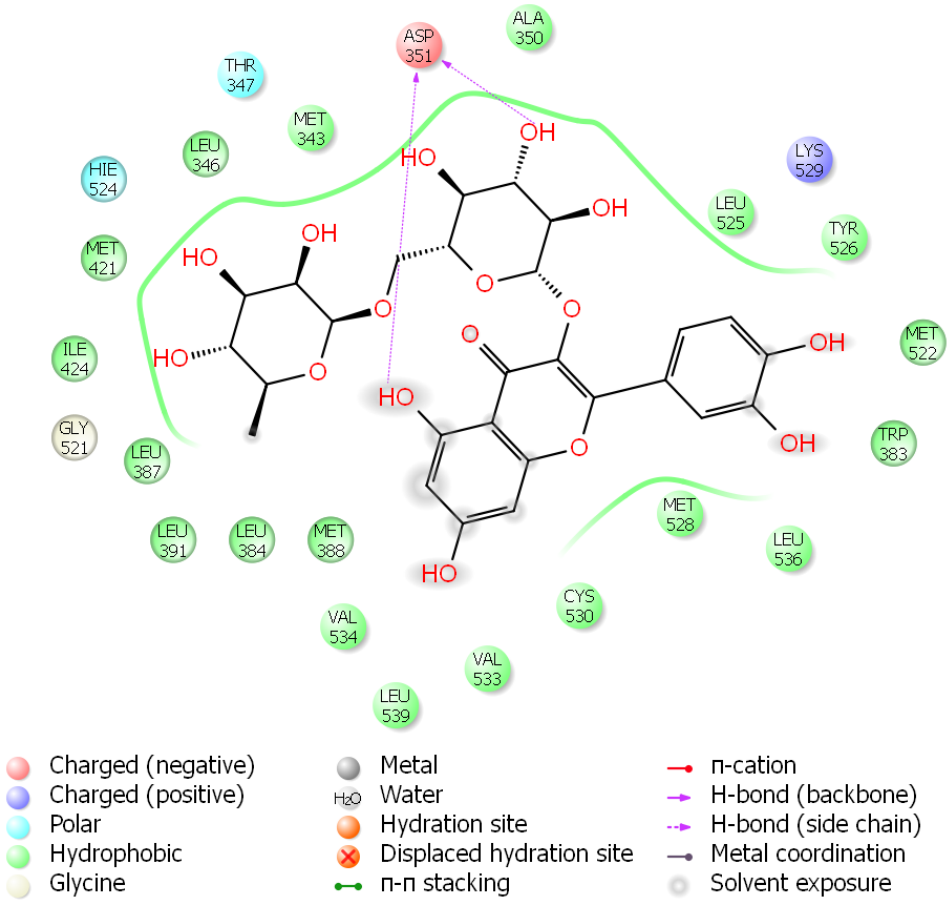


Figure 3: Ligand-recptor 2D interaction diagram of Rutin

3. Quercetin

Quercetin was seen as second top hit with the docking score of -9.60. The hydrogen bond interations of hydrogen and oxygen atoms of ligand with GLU 353 and ARG 394 were seen respectively. The other hydrophobic interactions observed were LEU 387, LEU 391, LEU 428, MET 388, ILE 424, LEU 384, MET 343, LEU 525, MET 528, TRP 383, ALA 350, LEU 346, and LEU 349 (Figure 4).

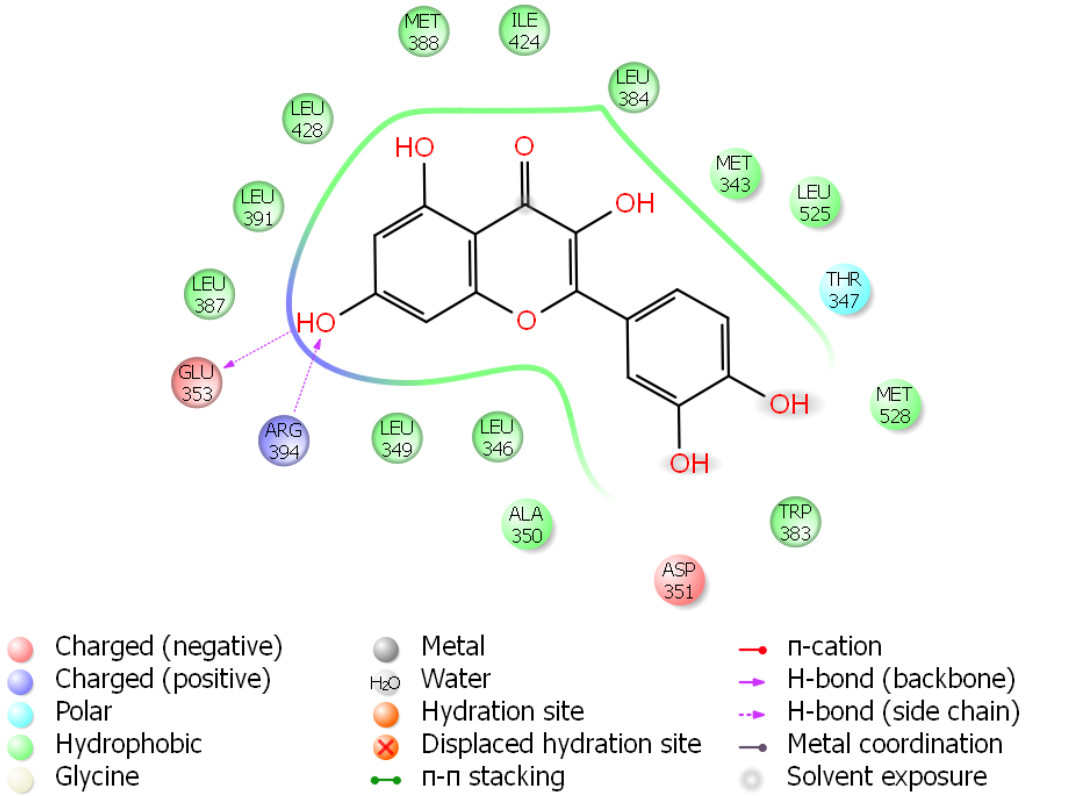


Figure 4: Ligand-recptor 2D interaction diagram of Quercetin

4. Myrecetin

Myrectin was the third top hit with the docking score of -9.34. The hydrogen bond interactions with two hydrogen atoms of ligands with GLU 419 and ASP 351 residues of receptor were observed. The other interactions seen were π-π stacking (TRP 383) and hydrophobic interactions of VAL 418, MET 343, TRP 383, LEU 525, LEU 354, ALA 350, LEU 387, LEU 384, MET388, LEU 428, LEU 346, PHE 404, LEU 391, ILE 424, and MET 421 residues of receptor with ligand.

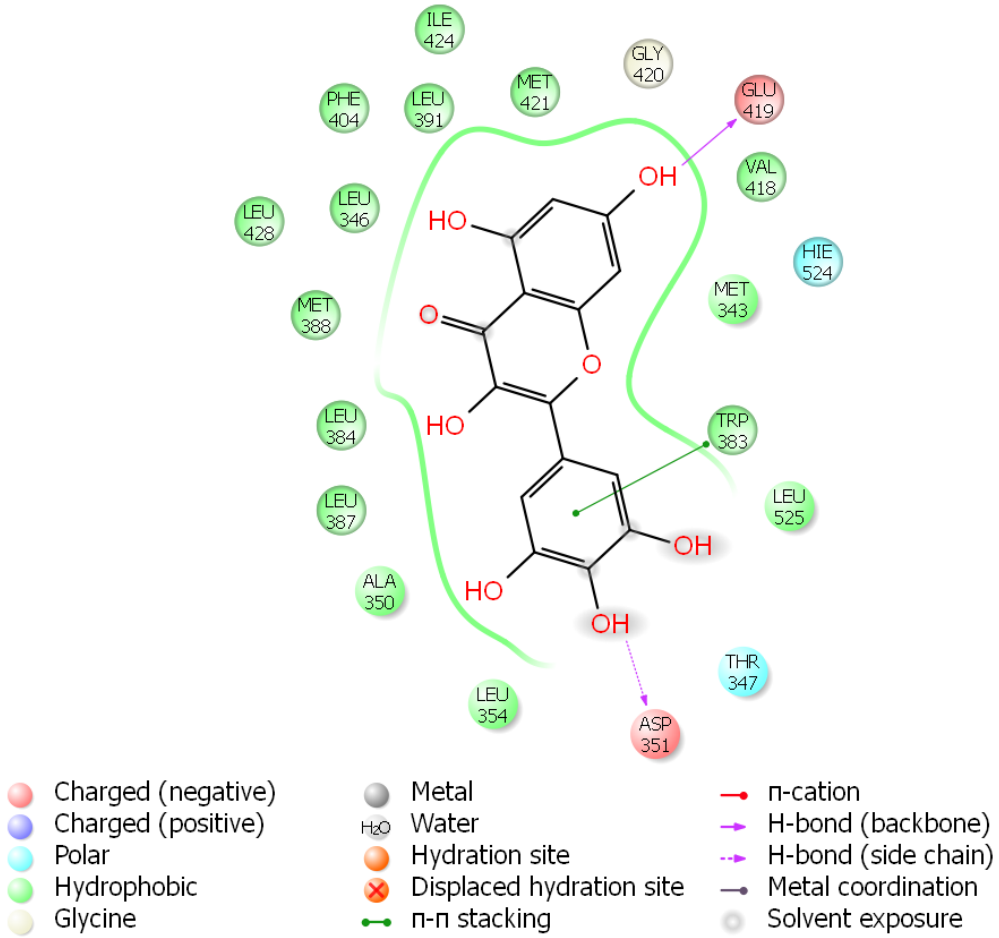


Figure 5: Ligand-recptor 2D interaction diagram of Myrecetin

CONCLUSION

During drug discovery, effective screening procedures could be applied to reduce cost and time. In this study; molecular docking has been used to analyze the binding ability of 29 compounds with the dihydrofolate reductase receptor. Ligands such as rutin, quercetin, myrecetin have shown good binding affinity with the protein (Figure 2 to Figure 5). This study throws a light on further experimentally validating these drug lead entities as microbial inhibitors.

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